

Delayed partial liquid ventilation shows no efficacy in the treatment of smoke inhalation injury in swine

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Harrington, D. T., B. S. Jordan, M. A. Dubick, L. C. Cancio, W. Brinkley, S. Kim, D. G. Burleson, A. Delgado, and C. W. Goodwin. Delayed partial liquid ventilation shows no efficacy in the treatment of smoke inhalation injury in swine. *J Appl Physiol* 90: 2351–2360, 2001.—In an earlier neonatal porcine model of smoke inhalation injury (SII), immediate postinjury application of partial liquid ventilation (PLV) had dramatic beneficial effects on lung compliance, oxygenation, and survival over a 24-h period. To explore the efficacy of PLV following SII, we treated animals at 2 and 6 h after SII and followed them for 72 h. Pigs weighing 8–12 kg were sedated and pharmacologically paralyzed, given a SII, and placed on volume-cycled, pressure-limited ventilation. Animals were randomized to three groups: *group I* (+SII, no PLV, $n = 8$), *group II* (+SII, PLV at 2 h, $n = 6$), and *group III* (+SII, PLV at 6 h, $n = 7$). Ventilatory parameters and arterial blood gases were obtained at scheduled intervals. The PLV animals (*groups II* and *III*) followed a worse course than *group I* (no PLV); PLV groups had higher peak and mean airway pressures, oxygenation index, and rate-pressure product (a barotrauma index) and lower lung compliance and arterial partial pressure of oxygen-to-inspired oxygen fraction ratio (all $P < 0.05$). PLV conferred no survival advantage. The reported beneficial effects of PLV with other models of acute lung injury do not appear to extend to the treatment of SII when PLV is instituted in a delayed manner. This study was not able to validate the previously reported beneficial effects of PLV in SII and actually found deleterious effects, perhaps reflecting the predominance of airway over alveolar disease in SII.

barotrauma; perfluorocarbon; pigs

SMOKE INHALATION INJURY (SII) accompanies thermal injury in up to 30% of patients admitted to burn centers. This injury, caused by inhaled cytotoxic chemicals and irritants, can cause severe ventilation and oxygenation abnormalities by its damaging effect on airways and air spaces (alveoli and distal bronchioles). Within 2 h of injury, large areas of the airways can be denuded and replaced by an inflammatory pseudomembrane, and within 6 h many air spaces are flooded with inflammatory debris and fluid (15). SII is associated with adverse outcomes, including increased rates of pneumonia and mortality. Mortality rates can be 20–60%

above those predicted by the extent of burn and age alone in patients with burns of moderate size (30–60% of the body surface area) (33).

The impact of inhalation injury on outcomes has led to experimental work in immune modulatory therapies, such as platelet-activating factor antagonists and pentoxifylline, and novel ventilatory adjuncts, such as inhaled nitric oxide and high-frequency ventilation and intravascular CO₂ extractors (25, 29, 43). Recent reports on the success of partial liquid ventilation (PLV) in both animal and human acute respiratory distress syndrome (ARDS) trials have led several investigators to evaluate PLV in the SII model. Perfluorocarbons are dense (1.9 g/ml), inert, and colorless fluids with excellent oxygen (50 ml/dl)- and CO₂ (210 ml/dl)-carrying capacity. PLV therapy involves intratracheal instillation of perfluorocarbon to functional residual capacity and then mechanical ventilation with standard ventilators. A 24-h PLV trial for inhalation injury performed at this institute compared neonatal pigs treated with PLV with pigs conventionally ventilated. The animals treated with a perfluorocarbon (perflubron, Liquivent, Alliance Pharmaceuticals, San Diego, CA) had decreased peak, plateau, and mean airway pressures, preservation of arterial pH, and significantly improved oxygenation and survival vs. the conventionally ventilated animals (7).

There are several possible mechanisms to explain the dramatic improvement in outcome seen in these trials. First, perfluorocarbon may act as a mechanical stent, which recruits and then maintains open distal airways and air spaces through expiration. This effect would allow continued gas exchange during all cycles of ventilation and eliminate repeated cycles of barotrauma as attempts are made to open those airways and air spaces. Avoidance of further injury in the form of either high airway pressures or infection has been shown to allow healing of the respiratory mucosa in 12–14 days (15, 39, 40). Furthermore, the weight of perfluorocarbon in the dependent areas of the lung may redistribute blood to nondependent, better-ventilated alveoli (10). Second, perfluorocarbon may act as an ideal lung lavage solution because it does not ap-

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pear to wash out surfactant. In the case of SII, perfluorocarbon could theoretically remove soot and desquamated cells and, in this and other models of acute lung injury, could remove proteinaceous debris from the distal air spaces (9, 11, 21, 35). Lastly, there is evidence that perfluorocarbon may act as an immunomodulating agent. Incubating neutrophils in perfluorocarbon causes cells to have impaired chemotaxis, phagocytosis, and oxidative burst (28, 38). Incubating alveolar macrophages in perfluorocarbon reduces H_2O_2 , superoxide, and cytokine production (34, 36).

Perfluorocarbon has been evaluated in many models with dramatic success. In a premature ewe model, Wolfson et al. (42), utilizing total liquid ventilation, and Leach et al. (18), using PLV, showed improved oxygenation and CO_2 clearance, improved compliance, and dramatically improved histology. A model of surgically induced, congenital diaphragmatic hernia in lambs by Major et al. (20) and Wilcox et al. (41) documented improved compliance, reduced wet-to-dry lung ratios, and short-term survival with perfluorocarbon therapy (perflubron, Liquivent, Alliance Pharmaceuticals). Nesti et al. (23) showed improved oxygenation, compliance, and histology at necropsy in a swine model of gastric aspiration using liquid ventilation. Hirsch and colleagues (12, 13), in an oleic acid-lung lavage model of ARDS, showed that liquid ventilation resulted in dramatic improvements in oxygenation, reduced shunt fraction, and improved CO_2 clearance and lung compliance. Richman et al. (27) showed similar improvements using perfluorocarbon as a lavage fluid. Two human phase I and II trials reported results with PLV in patients receiving extra corporeal life support. Both trials reported improved pulmonary compliance and oxygenation using PLV (14, 19).

Our previous study (7) revealed that PLV has the potential to decrease mortality at 24 h after inhalation injury; therefore, we embarked on a second trial of PLV in SII. Like most injury models evaluating PLV, our previous study treated animals within 1 h of injury. A patient with an inhalation injury rarely presents for medical care within 1 h of injury, and, often, the severity of the injury is underappreciated until the patient manifests signs of hypoxemia or high ventilatory pressures. Although our initial study animals treated with PLV showed remarkably stable oxygenation and ventilatory indexes up to 24 h postinjury, this period may not have allowed for the full clinical manifestation of inhalation injury, thereby providing an inadequate test of the efficacy of PLV (24). To explore the clinical efficacy of PLV and evaluate whether PLV protects the airway to a degree that allows for mucosal healing and long-term survival, animals subjected to SII were treated with perfluorocarbon at different times after injury and followed for 72 h. We felt that this evaluation would provide a suitable test of the efficacy of PLV in a clinical setting.

MATERIALS AND METHODS

Animal selection and model development. We used 8- to 12-kg female and castrated male pigs, rather than the 3- to

5-kg neonatal pigs used in the previous trial, to provide a hardier animal for the 72-h experiment. Model development focused on establishing an intensive care unit care and ventilatory algorithm for the 72-h trial and determining the smoke inhalation dose. Initial attempts to maintain animals without SII that were mechanically ventilated (tidal volumes of 13–15 ml/kg) and sedated for 72 h in dorsal recumbency were unsuccessful. These animals ($n = 5$) had dramatic decreases in oxygenation, increases in barotrauma indexes, and, at necropsy, lungs that were massively atelectatic. By the above parameters, these no-smoke, early model development animals followed a course similar to that of the animals that later received a moderate SII as part of the randomized trial. These differences were evident by 18–24 h after injury. The institution of larger tidal volumes (18 ml/kg) and repositioning the animal every 2 h ($n = 4$) from left to right lateral recumbency improved these parameters, as shown in the no-smoke, late model development animals (Fig. 1). Even with the positioning and increased tidal volumes, there was some deterioration of the animals' ventilation and oxygenation parameters due to the model. These changes were progressive but appeared to be most significant at 48 h and beyond (Fig. 1).

Experimental procedure. The experimental design was approved by the institutional review board and animal care and use committee. All surgeries were performed under aseptic conditions, and animal handling took place in an Association for Assessment and Accreditation of Laboratory Animal Care certified facility. The animals were fasted overnight, administered 0.02 mg/kg atropine sulfate (Eli Lilly, Indianapolis, IN), and subsequently anesthetized with a combination of intramuscular 0.05 mg/kg ketamine HCl (Ketalar, Parke-

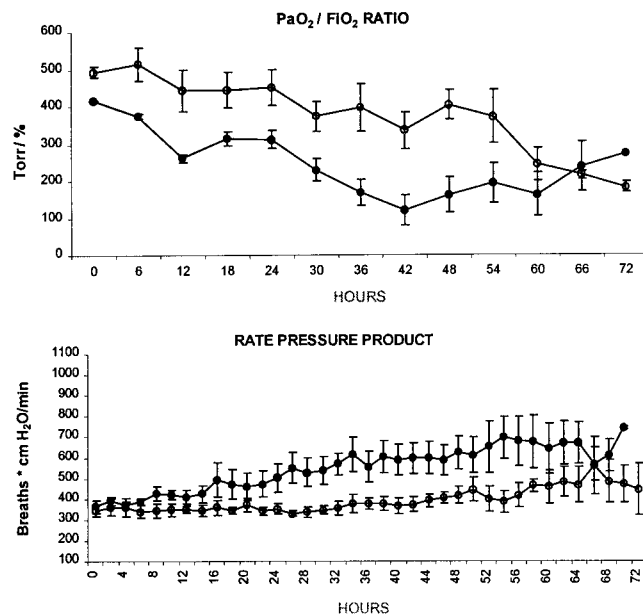


Fig. 1. The process of model development revealed that tidal volumes of 13–15 ml/kg and the dorsal recumbency position ($n = 5$) led to a deterioration of each animal's arterial PO_2 -to-inspired O_2 fraction ratio (PaO_2/FIO_2) and an increase in each animal's barotrauma score – rate-pressure product ($RPP = \text{ventilatory rate} \times \text{peak airway pressure}$) (●, early model development, no inhalation injury), which paralleled the changes later seen in the smoke inhalation injury (SII) groups in the randomized trial. Institution of higher tidal volumes and frequent repositioning ($n = 4$) resulted in a more stable clinical curve (○, late model development, no inhalation injury). Values are means \pm SE.

Davis/Warner Lambert Pharmaceuticals, Morris Plains, NJ) and 0.05 mg/kg xylazine HCl (Rompun, Miles Pharmaceuticals, Shawnee Mission, KS). Animals were then endotracheally intubated with a 5-mm-ID cuffed tube and placed in dorsal recumbency. Under isoflurane anesthesia, each animal had a 4-Fr double lumen, 15-cm cannula inserted into a femoral vein and a 20-gauge, 13-cm single lumen cannula inserted into the adjacent femoral artery using sterile technique. A 5-Fr pulmonary artery catheter was placed in the other femoral vein, and flow was directed into the pulmonary artery using sterile techniques. The animals also had a tracheostomy and a suprapubic cystostomy placed.

After placement of the catheters, the isoflurane was stopped, and the animals received 0.05 mg/kg fentanyl citrate, then were paralyzed using 1 mg/kg pancuronium, placed on volume-cycled mechanical ventilation (Siemens 900C, Siemens-Elma, Solna, Sweden), and physiologically monitored. All animals were then given a SII and placed on conventional ventilation. SII was administered using wood chips incinerated in a modified smoker, which delivered cooled and oxygenated smoke as previously described (24). The smoke exposure used was selected to result in 50% mortality at 72 h after SII (16, 31, 32). Initially, and for the first 2 h after SII, ventilator settings were as follows: ventilatory rate of 14, tidal volume of 18 ml/kg, inspired oxygen fraction ($F_{I_{O_2}}$) of 1.00, and positive end-expiratory pressure (PEEP) of 4 cmH₂O. After SII, the animals were then randomized into three groups: *group I* ($n = 8$) served as the untreated population; *group II* ($n = 6$) received 30 ml/kg intratracheal perfluorocarbon at 2 h postsmoke; and *group III* ($n = 7$) received 30 ml/kg intratracheal perfluorocarbon at 6 h postsmoke. All groups received volume-cycled, pressure-limited ventilation such that a peak airway pressure of >40 cmH₂O prompted a reduction in tidal volume and an increase in ventilatory rate while keeping the minute ventilation constant (maximum ventilatory rate in the treatment algorithm was 40 breaths/min). Normocapnia was not a treatment goal, and elevated arterial partial pressure of CO₂ (P_{aCO_2}) levels was tolerated. All animals were returned to an $F_{I_{O_2}}$ of 0.60 2 h after injury. $F_{I_{O_2}}$ was increased as needed over the course of the experiment to maintain oxygen saturations >90%. Inspiratory-to-expiratory ratios were kept at 1 to 2 for this trial. PEEP remained at 4 cmH₂O and was not titrated.

Intratracheal instillation of perfluorocarbon was performed as in our previous partial liquid ventilation trial (7). An air-tight endotracheal tube adapter with an indwelling infusion line was interposed between the ventilator circuit and endotracheal tube. The animal was placed in dorsal recumbency, and warmed (37°C) perfluorocarbon was instilled through the catheter. Periodically, as the instillation progressed, the animal was disconnected from the ventilator, and the presence of a meniscus was assessed. The initial filling required 15–20 min, and, once a meniscus was attained, the instillation of perfluorocarbon was stopped, even if the 30 ml/kg point had not yet been attained. Every 2 h, the presence of a meniscus was assessed with the animal in dorsal recumbency as outlined above for animals in *groups II* and *III*. If a meniscus was present, no perfluorocarbon was given; if no meniscus was present, perfluorocarbon was instilled to replace its evaporative losses (23). At 48 h postsmoke, residual perfluorocarbon was aspirated from the trachea, and the remaining perfluorocarbon was allowed to evaporate over the next 24 h.

Pancuronium (0.1–0.2 mg·kg⁻¹·h⁻¹), fentanyl citrate (0.01–0.02 mg·kg⁻¹·h⁻¹), and midazolam hydrochloride (0.1–0.3 mg·kg⁻¹·h⁻¹) were titrated intravenously for main-

tenance of anesthesia from the period immediately postsmoke to the end of the study. All animals were fed intragastrically with Ensure every 6 h during the study. Urinary output was monitored, and boluses of lactated Ringer solution (10 ml/kg) were used to maintain a urine output of 1 ml·kg⁻¹·h⁻¹ for the duration of the study period. The animals were repositioned every 2 h from right lateral recumbency to left lateral recumbency. No animals received antibiotics prophylactically, but animals with a sustained temperature >39.5°C received cephalosporin (20 mg/kg) intravenously every 8 h for the remainder of the study. At the end of 72 h, surviving animals were euthanized intravenously with pentobarbital sodium (25 mg/kg) and administered a potassium chloride bolus (20 ml of a 200 g/l solution) through an existing intravenous line.

Ventilatory and hemodynamic monitoring. Ventilatory parameters, including ventilatory rate, PEEP, and peak, mean, and plateau (30% hold) pressures, were noted on all animals every 2 h. Dynamic and static compliance and a barotrauma index termed the rate-pressure product (equal to peak airway pressure × ventilatory rate) were calculated from these values (6). Arterial blood gases for pH, arterial partial pressure of oxygen (P_{aO_2}), P_{aCO_2} , and percent oxy- and carboxy-hemoglobin saturation were obtained immediately and at 6-h intervals after SII. P_{aO_2} - $F_{I_{O_2}}$ (P-F) and oxygenation index (equal to mean airway pressure × P-F) were calculated every 6 h. Hemodynamic parameters, including heart rate and systemic and pulmonary blood pressures, were recorded every 2 h. Thermodilution cardiac outputs were obtained every 6 h.

Serum inflammatory markers. Animals had blood samples drawn through an existing central venous line at baseline and at 12, 24, 48, and 72 h postsmoke. The blood was centrifuged, and the supernates were stored at -70°C until analyzed. Plasma markers of tissue injury were assayed using standard clinical chemistry technique. Alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, creatine kinase, and creatinine were analyzed on a Hitachi 911 clinical chemistry analyzer (Boehringer Mannheim, Indianapolis, IN). Thiobarbituric acid reactive substances (TBARS), expressed as nanomoles of malondialdehyde per milliliter of plasma, were determined in the butanol phase as described by Naito et al. (22) using 1,1,3,3-tetraethoxypropane as standard. To determine the peroxidation potential of plasma, TBARS were assayed in the absence and presence of 0.25 M FeSO₄ (3). Ferric reducing ability of plasma (FRAP), as an index of its antioxidant status, was determined spectrophotometrically by the method of Benzie and Strain (2).

Tissue analysis. After euthanasia, the right lung cranial lobe was frozen and stored at -70°C for later assays of tissue myeloperoxidase, TBARS, FRAP, and oxidized and reduced glutathione concentrations. These measurements were indexed to total protein content of the specimens to control for the confounding presence of the perfluorocarbon (3). Lung samples were homogenized in 50 mM potassium phosphate buffer, pH 7.4. TBARS and FRAP were determined as above. Oxidized and reduced glutathione were determined enzymatically as described by Anderson (1). Myeloperoxidase activity was determined by a modification of the method of Trush et al. (37). Briefly, tissues were homogenized in 50 mM potassium phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide, pH 6.0. The homogenates then underwent three freeze-thaw cycles and sonification, followed by incubation at 60°C in a water bath for 2 h to extract myeloperoxidase and reduce interfering substances. Samples were then centrifuged at 10,000 g for 30 min at 4°C. Myeloperox-

idase activity was determined in the resultant supernatant using dianisidine as substrate. The remainder of the right lung was evaluated for gross weight, wet-to-dry weight ratio, and a blood-free wet-to-dry weight ratio. Lungs had not been perfused to wash out red blood cells, but their presence was accounted for by the method of Drake et al. (5). The left lung remained inflated at the peak airway pressure that the animal was on during the study (at time of death or 72 h), and two airway sections (proximal trachea, left mainstem bronchus) and three parenchymal sections (left apical, left lingular, left dorsal diaphragmatic) were processed for light microscopy using standard technique for hematoxylin/eosin staining. Sections were evaluated by three separate histopathologists blinded to treatment group using a predetermined standard histological grading scale (Table 1) (4, 15). These three scores were averaged for each animal at each area sampled. Additionally, a mean airway score, from the proximal trachea and left main bronchus, and mean parenchymal score, from the three parenchymal sections sampled, were calculated.

Flow cytometry. Venous blood was collected from groups I and III using EDTA as anticoagulant at 0, 12, 24, 36, 48, and 72 h postinjury. Group II did not have blood sampled for flow cytometry. Cell staining was performed by the addition of 100 μ l of whole blood to 12 \times 75-mm polystyrene tubes containing 1 μ g/ml of monoclonal antibodies (MAb). MAb were as follows: the biotinylated mouse anti-pig CD25 was provided as a courtesy by the USDA; the purified anti-pig CD4, CD8, and CD18 were purchased from VMRD (Pullman, WA); and the normal mouse IgG was purchased from Becton Dickinson (San Jose, CA). The MAb was incubated with the blood for 15 min at room temperature in the dark. Two milliliters of Hank's balanced salt solution containing 0.1% bovine serum albumin were added to each tube to wash out any nonbound MAb. Cells were spun in a centrifuge at 140 g for 5 min at room temperature. Supernatant was aspirated, and cell pellet was resuspended. The secondary antibody was added as 50 μ l of a goat anti-mouse FITC-labeled (1:40

dilution) antibody (Southern Biotech, Birmingham, AL). Cells were incubated for 15 min at room temperature in the dark. Cells were washed again as previously described. The Streptavidin-PE [(Becton Dickinson, San Jose, CA) 50 μ l of a 1:20 dilution] was added and incubated for 15 min at room temperature in the dark. Cells were centrifuged at 200 g for 5 min at room temperature. Supernatant was decanted, and tubes were blotted dry with a gauze pad. Samples were washed one time as previously described. The cell pellet was resuspended in 400 μ l of a 2% formaldehyde (PolySciences, Warrington, PA). Cells were placed on ice in the dark until flow cytometric analysis. Flow cytometric analysis was performed utilizing a FACSCalibur flow cytometer (Becton Dickinson). Data were analyzed using CellQuest software (Becton Dickinson).

Statistical analysis. Ventilatory parameters and serum injury and inflammatory markers were assessed by repeated measures ANOVA with post hoc analysis of factors found to be significant by repeated independent Student's *t*-tests with Bonferroni correction. Tissue assays of myeloperoxidase, TBARS, and FRAP were analyzed by ANOVA. Mean histological scores were analyzed by Student's *t*-tests. Survival curves were analyzed by Gehan's Wilcoxon test. Significance was accepted for all analyses at a type I error of $P < 0.05$. Statistical analysis package was Statistica 4.5 for Windows (Statsoft, 1993).

RESULTS

All animals appeared to receive a similar degree of injury, as measured by the percent carboxyhemoglobin (group I, 85.9% \pm 1.9; group II, 86.3% \pm 0.9; group III, 84.6% \pm 1.2). These carboxyhemoglobin levels returned to <5% in all groups by 6 h after injury. For the ventilatory parameters measured, all treatment groups were similar for the first 4–8 h after injury; however, both perfluorocarbon groups, groups II and III, then deteriorated over the remainder of the experiment, exhibiting higher peak airway pressures (group effect: $P = 0.027$; group effect over time: $P < 0.001$), plateau airway pressures (group effect: $P = 0.053$; group effect over time: $P < 0.001$), and mean airway pressures (group effect: $P = 0.009$; group effect over time: $P = 0.008$) (Table 2 and Fig. 2), which were \sim 20% greater than the nontreated group by 24 h postinjury. As stated previously, PEEP remained at 4 cmH₂O, and inspiratory-to-expiratory ratio was kept at 1 to 2. However, the animals that developed peak airway pressures of \geq 40 cmH₂O were treated with a lung protective strategy of lower tidal volumes and higher ventilator rates (Table 2). The rate-pressure product was elevated by 50% in the PLV animals over the nontreated group by 24 h and increased further over the 72-h period (group effect: $P = 0.014$; group effect over time: $P < 0.001$; Fig. 2). Groups II and III also had lower effective ventilation with higher CO₂ (group effect: $P = 0.053$; group effect over time: $P < 0.001$) and a resultant respiratory acidosis (group effect: $P = 0.042$; group effect over time: $P < 0.015$) when compared with group I (Fig. 3). Dynamic (group effect: $P = 0.063$; group effect over time: $P < 0.001$) and static compliance (group effect: $P = 0.041$, group effect over time: $P < 0.001$) deteriorated in the PLV groups and

Table 1. *Histological grading scale*

Grade	Description
<i>Airway</i>	
0	Normal
1	Mild segmental attenuation of epithelium
2	Marked segmental attenuation of epithelium, subepithelial edema, epithelium poorly preserved
3	Segmental epithelial loss, subepithelial edema, epithelium poorly preserved
4	Greater than 50% erosion of epithelium
<i>Parenchymal</i>	
0	Normal
1	Distended alveoli, straight or angulate thin alveolar septa, no inflammatory cells or alveolar histiocytes
2	A few inflammatory cells or a small focal area of inflammation, alveolar histiocytosis, minimal amount of alveolar edema, interlobular edema, thickened and tortuous alveolar septa with mild congestion
3	Multifocal area with inflammatory cells and/or edema in alveolar septa and in alveoli that affects <50% of the section
4	Diffuse inflammation and/or edema that affects >50% of the section

A histological grading scale used in our institute for grading the severity of smoke inhalation injury was modified slightly for this trial. Three independent pathologists, blinded to treatment group, read all slides using this scale.

Table 2. Lung protective strategy

	Hour 0	Hour 12	Hour 24	Hour 36	Hour 48	Hour 60	Hour 72
<i>Ventilator rate</i>							
Group I	14.0 ± 0.1	14.0 ± 0.1	16.5 ± 2.0	20.0 ± 3.9	16.7 ± 2.3	18.5 ± 2.6	20.1 ± 3.4
Group II	14.1 ± 0.1	19.9 ± 4.2	23.0 ± 5.8				
Group III	14.2 ± 0.1	21.6 ± 3.5	33.3 ± 4.9	32.7 ± 4.5	36.4 ± 2.4	36.5 ± 2.4	40.0 ± 0.1
<i>Tidal volume</i>							
Group I	184.2 ± 8.7	183.9 ± 8.0	167.6 ± 15.2	156.4 ± 21.0	162.0 ± 16.2	150.0 ± 16.4	149.8 ± 24.0
Group II	180.0 ± 21.7	157.0 ± 21.1	137.8 ± 36.7				
Group III	176.4 ± 8.0	132.6 ± 16.8	90.3 ± 20.0	87.4 ± 11.7	71.6 ± 2.3	69.8 ± 1.5	68.0 ± 0.1
<i>Peak airway pressure</i>							
Group I	23.3 ± 1.2	27.3 ± 2.1	29.8 ± 2.1	30.9 ± 2.4	32.9 ± 1.8	33.5 ± 1.4	37.8 ± 1.2
Group II	21.8 ± 1.2	34.4 ± 2.9	35.2 ± 2.9				
Group III	27.6 ± 1.3	36.2 ± 1.7	38.7 ± 2.7	40.1 ± 2.5	43.5 ± 3.6	41.0 ± 2.1	46.1 ± 3.1
<i>Plateau airway pressure</i>							
Group I	21.3 ± 1.3	23.6 ± 1.6	24.7 ± 1.9	26.2 ± 1.9	26.1 ± 1.6	27.6 ± 1.7	27.9 ± 2.1
Group II	19.4 ± 1.6	27.4 ± 2.6	26.0 ± 2.8				
Group III	25.4 ± 1.4	29.2 ± 1.0	33.4 ± 3.5	35.5 ± 3.1	39.6 ± 2.6	39.7 ± 1.8	41.5 ± 2.9

Values are means ± SE. The lung protective strategy employed in this protocol was pressure regulated, volume cycled with avoidance of higher pressures (defined as peak air pressure >40). Once this peak air pressure was obtained, tidal volumes were decreased and respiratory rates would increase to maintain the same minute volume. Ventilation rate and tidal volumes at 12-h intervals during the trial are shown. Peak air pressure and plateau air pressures for each group at these same intervals are shown.

was consistently one-half of that of the nontreated groups.

Additionally, the perfluorocarbon groups showed an increasing oxygenation defect, with lower PaO_2 (group effect: $P = 0.082$; group effect over time: $P = 0.011$), oxygen saturations (group effect: $P = 0.046$; group

effect over time: $P < 0.001$), and P-F (group effect: $P = 0.042$; group effect over time: $P < 0.008$) (Fig. 4). P-F in the PLV groups was roughly 50% of the untreated groups by 24 h after injury, with a calculated value consistently <200 from 48–72 h after injury (Fig. 4). The PLV groups also had a much higher oxygen index (two- to threefold) over the untreated group (group effect: $P = 0.003$; group effect over time: $P < 0.001$; Fig. 4).

The untreated group had a mortality rate of 37.5% over the course of this 72-h trial, reflecting the severity

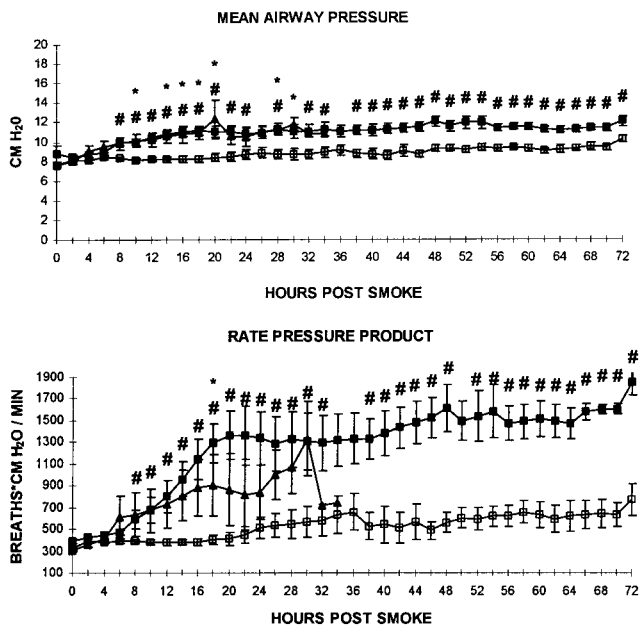


Fig. 2. Mean airway pressures and barotrauma, as measured by RPP (RPP = ventilatory rate \times peak airway pressure), were higher in the partial liquid ventilation (PLV) groups (\blacktriangle , group II; \blacksquare , group III) than the untreated group (\square , group I). This difference was evident within 6–10 h after SII and remained higher for the remainder of the experiment. Values are means \pm SE. Time points where there are significant differences ($P < 0.05$) between groups I and II (*) and groups I and III (#) are noted. Similar results were seen for peak and plateau (30% hold) airway pressures (data not shown).

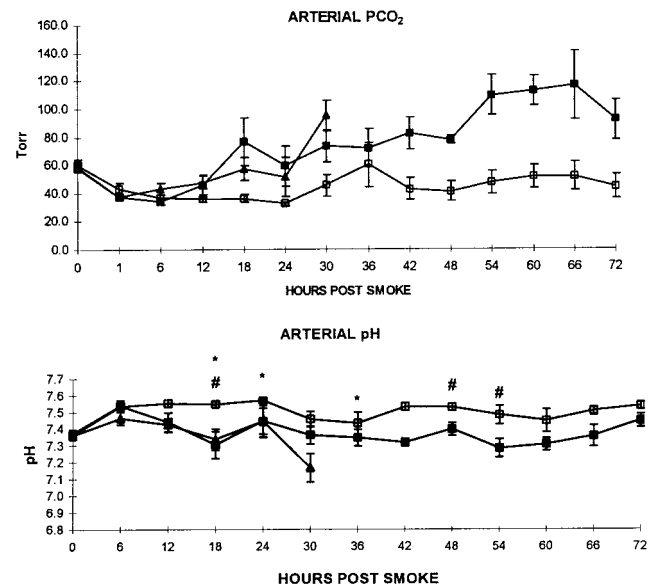


Fig. 3. PLV groups (\blacktriangle , group II; \blacksquare , group III) had a worsening respiratory acidosis with increasing CO_2 and a declining pH compared with the untreated group (\square , group I). Values are means \pm SE. Time points where there was a significant difference ($P < 0.05$) between groups I and II (*) and groups I and III (#) are noted.

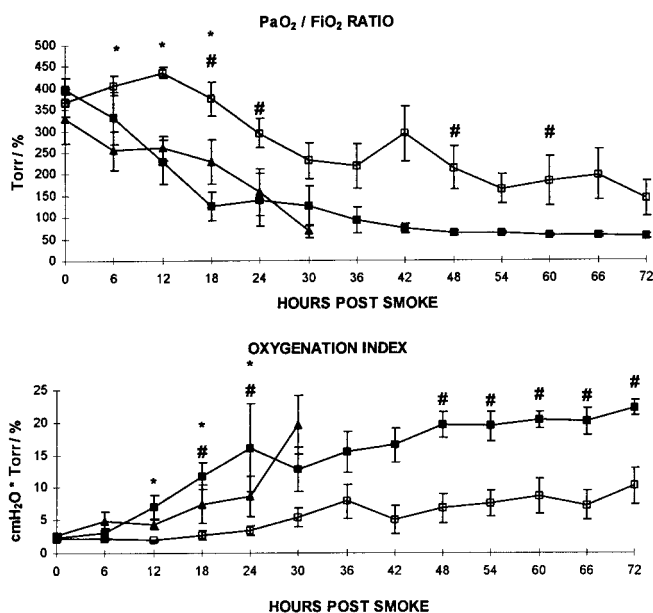


Fig. 4. PLV groups (▲, group II; ■, group III) had a decreased $\text{PaO}_2/\text{FiO}_2$ and higher oxygenation index, a sign of a significant oxygenation defect, compared with the untreated group (□, group I). The PLV groups had a $\text{PaO}_2/\text{FiO}_2$ of <200 by 24 h after injury. Values are means \pm SE. Time points where there was a significant difference ($P < 0.05$) between groups I and II (*) and groups I and III (#) are noted.

of the injury. Groups II and III had mortality rates of 100% and 57.1%, respectively. There was no statistical survival difference between groups I and III ($P = 0.207$). Group II, however, did have a statistically significant higher mortality than group I, with none of the group II animals surviving beyond 36 h ($P < 0.001$; Fig. 5).

No significant differences in plasma indexes of tissue injury (alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, creatine kinase, and creatinine) were observed among groups at any of the time points assayed. Perfluorocarbon treatment did not significantly reduce oxidative stress or lipid peroxidation following SII as measured by lung TBARS, antioxidant capacity, glutathione concentrations, or myeloperoxidase activity. In general, the lowest glutathione concentrations and highest TBARS and myeloperoxidase levels were observed in pigs treated with perfluorocarbon 2 h after SII compared with untreated animals, although, due to sample variance, the data did not achieve statistical significance. In addition, plasma TBARS, peroxidation potential, or antioxidant potential were also similar among groups at any time point when untreated and PLV groups were compared. Analysis of flow cytometry showed no decrease in the activation of circulating T cells, as measured by the presence of CD25 (IL₂R) on their cell surface.

Hemodynamic data were collected at 6-h intervals during the trial. Cardiac outputs ranged from 1.0 to 1.5 l/min. No significant difference in cardiac outputs existed between the treatment groups. Blood-free wet-to-dry lung ratios could not be obtained due to the inability

to separate perfluorocarbon from the lung after homogenization of the specimen. The gross weight of the specimens (right lung excluding the apical lobe), however, revealed that the lungs were heaviest in the perfluorocarbon groups (II and III): 114.9 ± 14.9 g for group I, 489.5 ± 103.2 g for group II, and 230.7 ± 40.1 g for group III. The differences among the groups were statistically significant ($P = 0.001$) and persisted when lung weights were controlled for body weights (Fig. 6). If this evaluation included only the animals that survived the entire trial, the perfluorocarbon animals in group III were still heavier than group I animals, although this did not attain statistical significance ($P = 0.125$; Fig. 6).

The two treatment groups received similar initial fills of perfluorocarbon (26.5 ml/kg for group II and 29.9 ml/kg for group III), yet they had different perfluorocarbon requirements to maintain a meniscus of perfluorocarbon at end expiration, with group II receiving on average $2.3 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ and group III receiving $1.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ($P < 0.001$). During the process of model development, animals treated with perfluorocarbon without prior inhalation injury ($n = 2$) required fills of 28.3 ml/kg on average and required $2.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ during the 72-h experiment. Their lungs at injury were lighter (119.5 g) at the end of the 72-h trial than the animals that had received SII and treatment with perfluorocarbon as noted above. These model development animals were remarkably stable throughout the

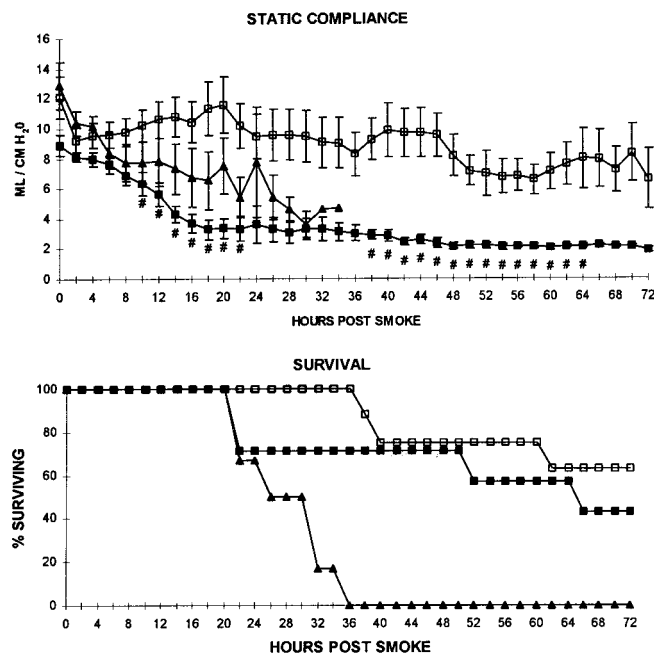


Fig. 5. Static compliance was lower in the PLV groups (▲, group II; ■, group III) than in the untreated group (□, group I) but was only statistically different between groups I and III. Similar results were found with dynamic compliance (data not shown). Groups I, II, and III had cumulative mortalities of 37.5, 100, and 57.1%, respectively. As a group, the PLV animals had a higher mortality rate than the untreated group (76.9 vs. 37.5%). Whereas the difference between groups I and III was not significant, group II had a statistically higher mortality rate than group I ($P < 0.05$).

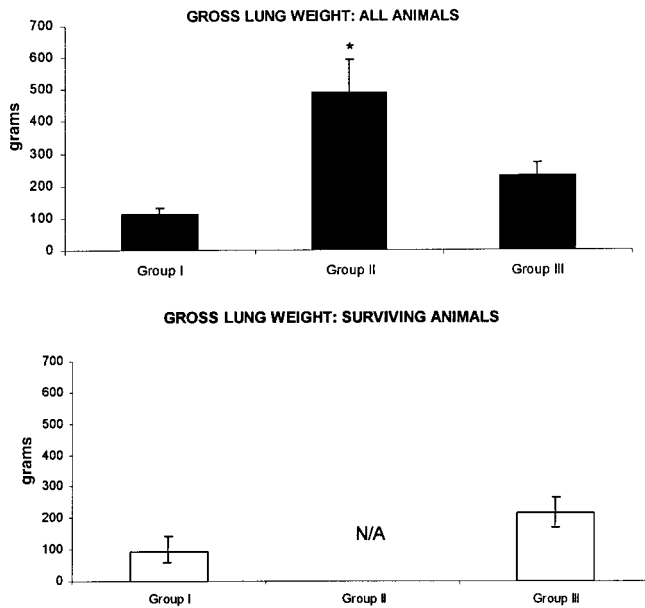


Fig. 6. Gross weights of the lungs of all animals at necropsy varied significantly among the groups, with the PLV being the heaviest. *Group II* had the heaviest lungs. Significant differences (* $P < 0.05$) were found between *groups I* and *II* but not between *I* and *III*. Gross weights of the animals surviving the entire trial (only *groups I* and *III*) showed a similar although not statistically significant trend. N/A, not available for *group II*; no animals survived 72 h. Values are means \pm SE.

72-h period, requiring no ventilatory manipulations with breath rates of 14 breaths/min throughout. Average peak airway pressures varied between 20 and 38, and average plateau airway pressures varied between 20 and 22 for the 72 h of the trial. Average P-F was >300 .

At necropsy, all groups had bronchial casts consistent with severe inhalation injury, which extended by visual examination to the second- and third-order bronchi. Most animals had patchy areas of lung collapse, but some animals had more severe areas of consolidation, with 50% of one of the lungs collapsed (2 of 6 for *group I*, 3 of 6 for *group II*, 5 of 6 for *group III*). No pneumothoraces occurred in the control group (*group I*), but *groups II* and *III* had several pneumo-

thoraces with free perfluorocarbon noted in the pleural spaces (2 of 6 for *group II*, 2 of 5 for *group III*). Two tension pneumothoraces occurred in the study, with both of these events occurring in *group II*. One of these animals had a rapid, sudden demise at 24 h after SII, and the second animal had reached the maximum breath rate of 40 breaths/min, and its peak airway pressures were allowed to climb after that to >40 cmH₂O.

Histological evaluations of all the animals in the randomized trial revealed a moderate to severe injury, with a mean airway score of 3.15 and a mean parenchymal score of 2.71. Although *group III* had a slightly better parenchymal score, there was no statistically significant difference between perfluorocarbon- and nonperfluorocarbon-treated animals at any of the areas sampled or in mean airway or parenchymal scores (Table 3). The three independent pathologists' readings showed good correlation, with no statistical significance detected among them at any of the five sampled areas by paired *t*-tests. During their evaluation of this experiment's histology, the panel of pathologists, using the same scoring scale and remaining blinded to both the experiment and treatment groups, also reviewed the previous microscopic slides from the 24-h neonatal pig PLV trial for inhalation injury at our institute. As in the present trial, there was no statistically significant difference in any of the sampled areas and mean airway or parenchymal scores between PLV and non-PLV groups. Although there were methodological differences between the two studies, a comparison between the non-PLV groups of both studies revealed that the current experiment appeared to have a slightly more severe injury as measured by histology. Mean airway scores were similar, but the present trial had a statistically higher mean parenchymal score (2.90 vs. 1.40, $P < 0.001$; Table 2).

DISCUSSION

Despite following our previous PLV protocol, the results of this trial of PLV in a severe SII stand in marked contrast to results of the animal and human ARDS trials. For almost every end point in this trial, the PLV-treated animals did worse and certainly were

Table 3. *Histological scores at necropsy*

	Air 1	Air 2	Par 1	Par 2	Par 3	Mean Air	Mean Par
Present trial							
Group I	3.33 \pm 0.21	2.69 \pm 0.34	2.95 \pm 0.36	3.04 \pm 0.46	2.71 \pm 0.44p	3.02 \pm 0.25	2.90 \pm 0.36
Group II	3.77 \pm 0.16	3.05 \pm 0.30	2.22 \pm 0.14	2.11 \pm 0.14	1.94 \pm 0.26	3.41 \pm 0.22	2.09 \pm 0.06
Group III	3.38 \pm 0.16	2.71 \pm 0.28	3.19 \pm 0.36	2.85 \pm 0.40	3.09 \pm 0.39	3.04 \pm 0.16	3.04 \pm 0.36
Fitzpatrick et al. (7)							
No PLV	2.46 \pm 0.66	3.53 \pm 0.16	1.40 \pm 0.41	1.40 \pm 0.51	1.40 \pm 0.50	3.00 \pm 0.39	1.40 \pm 0.47
PLV	1.86 \pm 0.38	2.80 \pm 0.32	1.27 \pm 0.31	1.27 \pm 0.29	1.33 \pm 0.26	2.33 \pm 0.30	1.28 \pm 0.27

Values are means \pm SE. In the current trial, histological scores provided by 3 pathologists were averaged for each treatment group at 5 areas of the left lung (Air 1, proximal trachea; Air 2, left mainstem bronchus; Par 1, left apical segment; Par 2, left lingual segment; Par 3, left dorsal diaphragmatic segment). Mean airway scores (Mean Air) for both the trachea and mainstem bronchus and mean parenchymal scores (Mean Par) for the 3 other areas were calculated. Partial liquid ventilation did not exert a beneficial effect on histology. Although the sampling in the current trial and the trial by Fitzpatrick et al. varied slightly, the areas of the Fitzpatrick trial that allowed a comparison between trials were reread by the same pathologists and are presented here. Comparison of the control groups *group I* of the present and *no PLV* in the Fitzpatrick trial shows no difference in mean airway scores but a worse parenchymal score in the current trial ($P < 0.05$).

no better than the untreated animals, which highlights the differences between SII and ARDS. Whereas there is a component of air space (alveolus and terminal bronchiole) injury in SII, the predominant pathological process in SII is an airway injury rather than an air space injury. After a moderate to severe inhalation injury, >50% of the tracheal mucosa is denuded, and large sections of the airway are covered with an inflammatory exudate that can extend into the distal bronchioles. This inflammatory process creates distal airway plugging that limits mixing of gases in spontaneously breathing and mechanically ventilated patients and may limit the free mixing of inspired gas and perfluorocarbon in PLV. Without this mixing, oxygenation and ventilation would deteriorate as non-ventilated perfluorocarbon becomes trapped in the distal airways. The heaviness of lungs in the PLV-treated animals is certainly compatible with a ball-valve mechanism, resulting in trapped nonoxygenated perfluorocarbon. Whereas overaggressive perfluorocarbon treatment might contribute to overfilling and trapping, in this study the animals received what is considered an optimal initial fill of ~30 ml/kg and received supplemental perfluorocarbon only if they did not have a meniscus present in their endotracheal tube (19, 26).

Recent evaluations of partial liquid ventilation protocols have found that optimization of PEEP by using levels nearer the lower inflection point of the pressure volume curve and inspiratory-to-expiratory ratios of 1 to 1 result in slight improvements of oxygenation and gas exchange (8, 17). Institution of these incremental improvements into our liquid ventilation protocol may have improved outcomes slightly in our PLV groups; however, a wealth of data has shown that the PLV protocol used in this and other trials was adequate and allowed for a reasonable test of PLV in SII.

The animal model could also be implicated as poorly tolerating PLV, but many ARDS animal trials have used the swine model. Our choice of ventilatory strategy might also be criticized. It was clear during model development that noninjured, nonperfluorocarbon animals developed massive atelectasis. We employed larger tidal volumes and position changes to maintain open air spaces, which to a large degree worked well. We also employed a lung protective strategy of low tidal volumes once airway pressures increased (peak >40 cmH₂O) and followed a strategy of permissive hypercapnia. Other lung protective strategies such as optimal PEEP or pressure-cycled ventilation could also have been used, but we pursued a pressure-limited, volume-cycled strategy. Tidal volumes were not decreased after installation of perfluorocarbon. Our experience with our previous perfluorocarbon trial led us to believe that this change in tidal volume was not necessary. We noted no increase in airway pressures after filling the lungs to functional residual capacity in our previous and current trial. During our model development phase, animals without inhalation injury treated with PLV ($n = 2$) for 72 h were easily ventilated, with preserved oxygenation and ventilation and without the development of dense, perfluorocarbon-saturated lungs.

The stability of these two animals treated without smoke and perfluorocarbon argues that the differences seen between the treatment groups were the effect of the perfluorocarbon in the setting of inhalation, not the ventilatory strategy or perfluorocarbon protocol used.

The proposed effect of perfluorocarbon as an ideal lavage solution was not specifically addressed in this trial. A previous trial evaluating perfluorocarbon lavage vs. saline lavage in SII revealed that, whereas intermittent perfluorocarbon lavage eliminates the transient hypoxemia associated with saline lavage, it does not have a lasting effect on oxygenation (4). Furthermore, it was no more effective at removing cellular or proteinaceous debris than saline. These findings should not be surprising given perfluorocarbon's low miscibility with other substances. The dense inflammatory peel that is established in inhalation injury and is the driving pathophysiological process of inhalation injury appears resistant to perfluorocarbon lavage or PLV (32).

Perfluorocarbon's ability to act as an immune modulator is unclear. Our trial did not find any antioxidant or anti-inflammatory effects of perfluorocarbon either locally or systemically. Tissue levels at necropsy and plasma levels at scheduled time points during the trial showed no beneficial trends in TBARS levels, glutathione concentrations, or total antioxidant capacity following intratracheal perfluorocarbon. One marker of systemic activation following thermal injury is an increased level of activated T cells as measured by expression of CD25 (IL₂R) (30). In our trial, PLV therapy did not decrease the expression of IL₂R in circulating lymphocytes.

This trial's inability to validate the previously reported beneficial effect of PLV in SII demands further explanation. The rapid and sustained deterioration of the PLV groups in the present trial, which were not seen in the previous SII and PLV trial, argues that there were significant differences between models. Fitzpatrick et al. (7) used 3- to 5-kg nonweaned neonatal pigs. These animals may have been more prone to atelectasis than the 8- to 12-kg animals used in the present trial, suggesting that the positive results of their trial may have been the ability of PLV to treat atelectasis. During the early model development phase for this trial, animals ventilated with tidal volumes of 13–15 ml/kg (volumes used in the Fitzpatrick et al. trial) developed massive atelectasis, worsening hypoxemia, elevated airway pressures, and, frequently, early demise. Increasing tidal volumes to 18 ml/kg and repositioning of the animals every 2 h, methods adopted for this trial, prevented these sequelae. Also, this trial appeared to have a more severe inhalation injury with higher mean carboxyhemoglobin (86 vs. 47%) and grade of inhalation injury by histology. Although this histological picture could be explained by the different durations of the trials (72 and 24 h), even the animals that died <30 h after smoke were noted to have severe airway and parenchymal injury. Previous evaluations of morphological and histological changes following inhalation injury have demonstrated that there are

well-established light microscopic changes within 2–6 h of injury. By 2 h, the airway shows large areas of denudation, marked proteinaceous debris and the development of an inflammatory peel. By 6 h, the air spaces show thickened septa and alveolar flooding (15). The lack of efficacy even in the 2-h fill groups (a time course closer to that used in the earlier trial) appears consistent with the early development of an inflammatory reaction following SII described by numerous authors and found in our present trial. The 2-h treatment group appeared to do worse than the 6-h treatment groups and had dramatically heavier lungs at the conclusion of the trial, suggesting that perfluorocarbon in the early phase of airway inflammation is more prone to becoming trapped in distal air spaces behind a developing inflammatory exudate. The rapid development of inflammatory changes may render PLV ineffective except when used immediately after injury, where it may limit the development of the inflammatory cast of the airway. Immediate treatment is usually impractical, however, and severely limits PLV's clinical relevance in SII.

In conclusion, the model of inhalation injury evaluated in this trial was a more severe injury than that in the previous study (7) from this institute and is therefore a better test of PLV in the setting of SII. It appears that the presence of airway disease prevents the efficacy of this agent. This trial's study hypothesis was that PLV would have beneficial effects when instilled even 6 h after injury and that this effect would be sustained over 72 h. Our results showed that in our 8- to 12-kg swine model of SII, PLV-treated animals showed decreased oxygenation, worsened ventilation, higher airway pressures, and, in one group, higher rates of mortality. Further work is required to determine the role of PLV in the treatment of SII.

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